

Qubit™ ssDNA Assay Kit

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ ssDNA Reagent (Component A)	250 µL	200X concentrate in DMSO	<ul style="list-style-type: none"> • Room temperature • Desiccate • Protect from light 	When stored as directed, kits are stable for 6 months.
Qubit™ ssDNA Buffer (Component B)	50 mL	Not applicable	<ul style="list-style-type: none"> • Room temperature 	
Qubit™ ssDNA Standard #1 (Component C)	1 mL	0 ng/µL in TE buffer	<ul style="list-style-type: none"> • ≤4°C • Do not freeze 	
Qubit™ ssDNA Standard #2 (Component D)	1 mL	20 ng/µL in TE buffer		

Introduction

The Qubit™ ssDNA Assay Kit for use with the Qubit® 2.0 Fluorometer makes DNA quantitation easy and accurate. The kit includes concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is accurate for initial sample concentrations from 50 pg/µL to 200 ng/µL providing an assay range of 1–200 ng. The assay is performed at room temperature, and the signal is stable for 30 minutes. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix, Table 2*). In addition to the Qubit™ ssDNA Assay Kits described here, we also offer other kits for assaying RNA, protein, and dsDNA (*Appendix, Table 3*).

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ ssDNA Assay Kit

The Qubit™ ssDNA reagent and buffer are designed for room temperature storage. The Qubit™ ssDNA reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the ssDNA standards at 4°C.

Critical Assay Parameters

Assay Temperature

The Qubit™ ssDNA assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28°C). The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, store the Qubit™ ssDNA reagent and the Qubit™ ssDNA buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this warms the solution and results in a low reading.

Incubation Time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 30 minutes at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence is observed as the solution increases in temperature (see *Appendix*, Figure 2). Note that the temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 30 minutes. See Figure 1 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

No data are currently available addressing the mutagenicity or toxicity of the Qubit™ ssDNA reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ ssDNA reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol

The protocol below assumes you are preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you need fewer tubes (step 1.1) and less working solution (step 1.3). You can find more detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6, below) in the user manual accompanying the instrument.

- 1.1 Set up the required number of 0.5 mL tubes for standards and samples. The Qubit™ ssDNA assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Invitrogen Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part number 10011-830).

- 1.2 Label the tube lids.

- 1.3 Make the Qubit™ working solution by diluting the Qubit™ ssDNA reagent 1:200 in Qubit™ ssDNA buffer. Use a clean plastic tube each time you make Qubit™ working solution. **Do not mix the working solution in a glass container.**

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

- 1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

- 1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ ssDNA standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards are introduced to the instrument in the right order.

- 1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

- 1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

- 1.8 Allow all tubes to incubate at room temperature for 2 minutes.

- 1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **ssDNA** as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® Fluorometer* above for calibration guidelines.

- 1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.
- 1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.

Remove Standard #1.

Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

Remove Standard #2.

- 1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
- 1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

- 1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.
- 1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ ssDNA assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:

QF value = the value given by the Qubit® 2.0 Fluorometer

x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (*i.e.*, if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation will be in ng/mL).

Dilution Calculator The “Dilution Calculator” feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

- 2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.
- 2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.
- 2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.
- 2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/μL, the button will display pg/μL).

- 2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved in the .CSV file and tagged with a time and date stamp.
- 2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

How the Qubit® 2.0 Fluorometer Calculates Concentrations

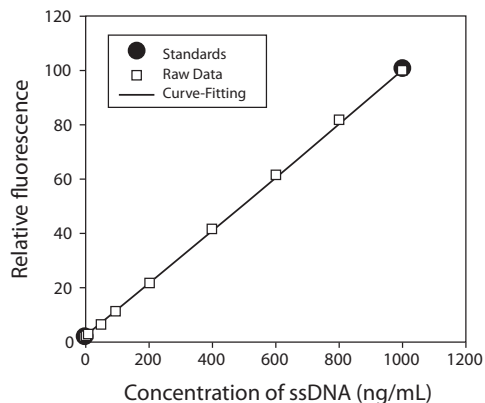


Figure 1. The curve-fitting algorithm used to determine concentration in the Qubit™ ssDNA assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ ssDNA assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ ssDNA Assay

Table 2. Effect of contaminants in the Qubit™ ssDNA assay.

Compound	Maximum Acceptable Concentration	% Signal Change*
Salts		
Ammonium acetate	50 mM	13% decrease
Sodium acetate	30 mM	3% decrease
Sodium chloride	100 mM	25% decrease
Zinc chloride	1 mM	43% decrease
Magnesium chloride	5 mM	34% decrease
Urea	2 M	47% increase
Organic Solvents		
Phenol	0.2%	19% decrease
Ethanol	10%	19% increase
Chloroform	2%	2% increase
Detergents		
Sodium dodecyl sulfate	0.01%	73% increase
Triton® X-100	0.1%	11% increase
Proteins		
Bovine serum albumin	2%	20% increase
IgG	0.1%	37% decrease
Other Compounds		
Polyethylene glycol	1%	29% increase
Agarose	0.1%	8% increase
ATP	0.1%	30% increase

*The compounds were incubated at the indicated concentrations with Qubit™ ssDNA assay in the presence of 660 ng/mL of a 24-mer M13 sequencing primer.

**Qubit™ Assay Kits Compatible
with the Qubit® 2.0
Fluorometer**

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	<ul style="list-style-type: none"> core range (high confidence): 0.01 µg/mL to 5 µg/mL† extended range (moderate confidence): 5 µg/mL to 10 µg/mL† useful for quantitation of genomic and miniprep DNA samples accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	<ul style="list-style-type: none"> core range (high confidence): 1 ng/mL to 500 ng/mL† extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† useful for quantitation of PCR products, viral DNA, and samples for subcloning accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	<ul style="list-style-type: none"> core range (high confidence): 5 ng/mL to 1,000 ng/mL† extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† useful for quantitation of oligos, primers, denatured DNA, PCR products accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	<ul style="list-style-type: none"> core range (high confidence): 25 ng/mL to 500 ng/mL† extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	<ul style="list-style-type: none"> core range (high confidence): 0.1 µg/mL to 5 µg/mL† extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	<ul style="list-style-type: none"> core range (high confidence): 1.25 µg/mL to 25 µg/mL† extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† little protein-to-protein difference in signal accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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